### Annual Report 2020/21



Eastern Canada Oilseed Development Alliance Prepared by: Treasa Pauley, ECODA Project Manager



## ECODA UPDATE

### Vision

Facilitating oilseed supply chain partnerships to drive innovation and capitalize on economic value.

### Mission

Profits through partnerships and innovation.

The **Eastern Canada Oilseeds Development Alliance (ECODA)** is a private, not-for-profit company based in Charlottetown, Prince Edward Island (P.E.I.). Formed in 2009, ECODA has created a consortium of reputable supply chain collaborators within the Canadian oilseeds industry, spanning soybeans, canola, and novel crops. ECODA aims to benefit oilseed growers, processors, and exporters in Eastern Canada by establishing oilseed crop options that match regional factors and market needs and supporting innovative development throughout the oilseed value chain. When one link in the chain finds success, the rest can benefit as well, resulting in full value chain prosperity.

ECODA's current round of funding includes support through the federal AAFC Canadian Agricultural Partnership (CAP) AgriScience Projects program as well as provincial support from the New Brunswick Department of Agriculture, Aquaculture and Fisheries and the Prince Edward Island Department of Agriculture and Fisheries under their respective CAP funds. The current research program includes multiple AAFC researchers and research stations, academic institutions, private sector research organizations, grower, and industry partners from across Canada as well as international contributions from Europe, Japan, South America, and the United States. The ECODA CAP research program has focused its research on sustainable supply chain development of soybeans, canola, brown mustard, camelina, and pulses.

We are well into our fourth year of our five-year program, which has been nothing short of an adventure. Despite climate change impacts on seasonal temperatures and moisture conditions and a global pandemic our excellent team of researchers and collaborators have endured. 2020 was a year of some adjustments with researchers having to modify processes and procedures to ensure the safety of all involved, many completing work with skeleton crews of staff. AAFC made contingencies for the pandemic impact on research and for the first-time permitted amendments to budgets not previously allowed. This provided some relief to researchers as restrictions impeded overall annual deliverables not just to field activities but also lab analysis and especially when international work was involved.

The ECODA program saw some reductions as well as some additions to its program this year. Due to diminishing market potential and the resulting shift of collaborator priorities it was decided that the anticipated hemp investigations would be abandoned. ECODA maintains its mandate to support commercially relevant research and there simply was not regional support for this endeavor given the shift in market since 2018. 2020 did see growth however in the canola research program with the addition of proteomics evaluations within Dr. Smith's activities. This support allows us to delve deeper into the plant-microbe signaling relationships that show potential for improved plant adaptations to climate change stressors.

As this round of funding approaches its final year, we not only take a deeper look at what we have learned but also prepare and strategize for the future. The challenges of the past three and a half years have made a few things abundantly clear, bringing to light key areas that need to be addressed for agriculture in Canada to survive and thrive. The first of these is climate change is here. There is no longer a "normal" season that fits our expected or anticipated growth conditions. We need to address this in our cropping systems for the future, growing crops and developing varieties that are adaptive to the various stressors while still of economic value to the current market demands. The other side to this is also looking at how agriculture is impacting this environmental phenomenon and ensuring that we are implementing sustainable practices for the future of the industry. Another lesson which was felt across the country during the height of the global COVID pandemic was food security. We as a nation must look at our food and feed supply chains and examine where we can support the gaps internally. It is no longer sustainable for Canada as a nation to rely on commodity production that we then export and buy back at higher value finish products. We need to start investing time and money into our own innovation and value adding.

Preparing for the next round of investment, project managers are reaching out to researchers and collaborators to discuss future supply chain priorities and opportunities. We encourage all who have innovation to present to get in touch with one of the ECODA administrators to discuss projects and proposals.

As this round of research projects approach the final year, we are making a strong effort to increase the tech transfer output to ensure the efforts of our researchers are being disseminated to industry. COVID restrictions has invited much creativity in digital information development and sharing. The most recent KTT project has been the creation of a video series (YouTube – ECODATV) that highlights some of our researchers and their innovative projects. There are presently 3 videos available for viewing online with a fourth planned for early fall. Please, if you have not already done so, follow, like and share the ECODA pages (Twitter: @CanadaOilseeds, Instagram: easterncanadaoilseeds) so we might get the message out.

# **CANOLA REPORT**

### Plant-microbe Interactions to Overcome the Negative effects of Stress and Enhance Canola Yields

#### Principal Investigator: Donald L. Smith, McGill University

Use of biologicals in agricultural systems can reduce the environmental impacts of various stressors, including those associated with climate change (drought, high temperature). The application of plantassociated microbes, and/or the signal compounds known to be produced by these microbes, can increase plant resistance to these types of stress and improve the adaptability of the crop. Thuricin 17 was identified as a microbe-to-plant signal produced by a *Bacillus thuringiensis* strain isolated from soybean nodules in southwestern Quebec. It has been shown to enhance plant growth of many crop species when the plants are under stress. However, it has not been evaluated for mitigation of stresses for canola, a key Canadian crop. These stresses include low temperature, drought, as well as the combination of high temperature and drought. The ECODA research seeks to address this. Much of the research to date has focused on the first phase of plant growth, seed germination, and has examined low temperature stress, the stress likely to be encountered by germinating seeds under field conditions. The work has shown that treatment with thuricin 17 enhances both germination velocity and the proportion of seeds that successfully germinate, under low temperature stress.

The ECODA research is being conducted in several parts, controlled environment studies, field studies, and proteomics work, which was added in 2020. Proteomics is used to identify and quantify protein expression at a given growth point in response to a specific stimulus. The proteomics study will assess the utility of thuricin 17 in improving canola growth in the presence of two stress conditions: drought and heat. The two most effective thuricin 17 concentrations will be determined through canola germination tests. The best concentration(s) and level(s) of drought will be applied for proteomic studies. Leaves will be sampled, extracted for proteins, and sent to the Institut de Recherches Cliniques de Montréal (IRCM) for proteomic analysis.

#### Results to date:

**2020** field data has indicated that the effect of foliar spray-applied thuricin 17 on yield, harvest index and pod number was statistically significant on clay-loam and sandy-loam soils at the first seeding date. This thuricin 17 treatment enhanced yield by 19 and 17%, respectively, compared to the control. Plots treated with the foliar treatment of thuricin 17 had the highest harvest indices and led to increases in leaf area of 14 and 83%, for clay-loam and sandy-loam soils, respectively, and biomass weight at flowering of 11 and 65%, respectively. These responses make increased yields reasonable

as higher leaf area causes more photosynthesis, more carbohydrate production, and finally leads to higher yield. The second seeding date showed no statistically significant increases in yield on either soil type which may indicate that thuricin 17 works better under more stressful conditions. The first seeding was more stressful for plants than the second due to an early period of very hot and dry conditions.

It is difficult to arrive at any precise conclusions from this single year of field data, regardless of the four site years established as considerations need to be made for the late start date due to the COVID19 pandemic. This resulted in the canola plants facing difficult climatic conditions during seedling establishment, which was particularly so for the early seeding date trials.

Early 2021 has been spent starting the high temperature stress experiments in a growth chamber and data will be collected on the ability of thuricin 17 to enhance canola vegetative growth under high temperature stress conditions.

Studies on thuricin 17 have illustrated its promising role as a plant growth stimulator under stressful conditions and our initial results agree with previous research that showed improved growth and development, under stressful conditions compared with control plants in either greenhouse or field conditions.

#### 2021 Progress:

The second year of field trials are being conducted as planned and activity progresses with minimal impact as COVID-19 continues.

# Specialty Canola Germplasm with Clubroot Resistance for Eastern Canada

#### Principal Investigator: Sally Vail, AAFC, Saskatoon, Saskatchewan

The goal of this research is to develop non-GMO specialty canola varieties with resistance to Clubroot for Eastern Canada. Lack of these types of canola varieties has reduced capacity to produce GMO-free canola seed and oil on a competitive scale. As a result, an industry which could easily exceed a value of \$2M, if Clubroot Resistant (CR) GMO-free varieties were available, is currently hampered.

There is potential of a larger export market of GMO-free specialty canola oil from eastern Canada, mainly the province of PEI. However, around 2010, the disease clubroot became a problem in some canola fields, significantly reducing yields. Currently there are no commercially available clubroot resistant, non-GMO canola varieties available for growers in this region. Thus, AAFC is developing non-GMO, specialty canola lines with clubroot resistance through the ECODA CAP project. Unfortunately, due to Covid-19, several activities in this project were reduced or delayed in 2020. Yield trials in the Maritimes and Saskatchewan proceeded, however the development of new germplasm was severely hampered when greenhouse and laboratory activities were stopped in March 2020. Fortunately, activities which will contribute to deliverables in Objectives 2 & 3 of this project resumed in the fall of 2020. As a result, several deliverables were shifted in this project.

#### **Results to Date:**

Successful yield trials were conducted at three sites in Saskatchewan and one location in Nova Scotia in 2020. A candidate line of interest for possible registration was grown by a producer in PEI over the summer of 2020 to assess on-farm yield potential. In Saskatoon, isolated increases of two lines, one black seeded and the other yellow seeded, were performed and yielded good quality seed for future use in this project. The PEI clubroot nursery was cancelled in 2020 due to Covid-19 restrictions.

When the candidate black-seeded lines were examined, yield of VR234 and VR233 were the highest yielding combined with optimal seed oil content. The seed yield matched or exceeded that of the recurrent, black-seeded parent (N99-508) and was 79% and 74%, respectively, of what was obtained off the check commercial hybrids. Both lines contain clubroot resistance gene *Rcr1* and showed no gall formation in the PEI clubroot nursery in 2018 and 2019. Based on initial yield trial and seed quality results, VR250 was the line that was initially selected for seed increase. In an increase performed in Saskatoon in 2020 yielded approximately 450 Kg of high-quality seed which is available for further increases if there a market for this line as a registered variety.

The top yielding yellow-seeded lines across seven yield trials is VR16-517 and VR16-522, both which contain *Rcr2* and have no exhibited galls in the 2018 or 2019 PEI clubroot nurseries. Agronomic characteristics of these lines are very similar to the recurrent parent YN01-429 with exceeding yields that are 92% and 91% of the hybrid checks, respectively. Oil content of the yellow-seeded lines is very high with values exceeding that of the commercial hybrid checks by almost 3%. The seed protein content of the lines is about 2.5% lower than the commercial checks, which is a consideration for varietal registration of these lines, depending on regional

requirements. Approximately 300 Kg of VR17-053 seed was produced in an increase in Saskatoon in 2020, which was the line that was looking most promising after the initial year of yield trials in 2018.

#### 2021 Progress:

Trials are being conducted as planned along with the additional modifications from 2020 amendments.

### Occurrence of Swede Midge and Evaluation of Potential Control Mechanisms in Canola Production in Ontario and Quebec

#### Principal Investigators: Rebecca Hallett, University of Guelph & Sebastien Boquel, CEROM

The swede midge (SM), *Contarinia nasturtii*, is an invasive pest of cruciferous crops that can negatively impact canola production in Ontario and Quebec. To improve SM management in canola, we are investigating the biological control potential of *Synopeas myles*, a parasitoid of SM. Relatively high rates of SM parasitism by *S*. *myles* in the field have been observed in the past years, suggesting that *S. myles* is well established in Ontario and Quebec. However, before *S. myles* can be incorporated into an IPM program for SM, its abundance, distribution, potential to suppress SM, and compatibility with other IPM tactics must be determined.

The overarching goal of this research is to gain knowledge about the swede midge (SM) in Ontario and Quebec and investigate the potential for biological control agents to improve management of this devastating pest of canola.

#### **Results to Date:**

#### Quebec

Each year, the Québec IPM Network (*Réseau d'Avertissements Phytosanitaires;* RAP), tracks SM populations in several canola fields in the main canola growing regions of Quebec. In 2020, 29 canola fields were selected in five regions: Abitibi-Témiscamingue (AT), Saguenay-Lac-St-Jean (SLSJ), Capitale-Nationale (CN), Chaudière-Appalaches (CA), and Bas-St-Laurent (BSL). Information on management and agricultural practices, as well as field characteristics, were gathered for each field. SM populations in each field were monitored using pheromone traps. SM damages were evaluated twice during the season – at bolting and at the beginning of ripening. For each plant, all racemes were inspected for symptoms of infestation. The plant was deemed healthy if none of the racemes showed signs of infestation. If at least one raceme showed signs of infestation, the plant was considered damaged. For each trap, a catch rate (number of SM/trap/day) was calculated for the period preceding the first evaluation and another one for the period between the first and the second evaluation. The percentage of plants showing symptoms of infestation was then correlated to the catch rate preceding the evaluation.

The overall SM catch rate (SM/trap/day) for all fields followed by the RAP in 2020 is presented in **Figure 1**. Interestingly, the abundance of SM in the site located in CN has increased over the years, 0.1 SM/trap/day in 2018, 1.1 SM/trap/day in 2019 and 10.6 SM/trap/day in 2020. That is a 10-fold increase every year.



### **Figure 1.** Swede midges catch rate (number per trap-day) in the fields surveyed by the *Réseau d'avertissements phytosanitaires* across different regions of Québec in 2020.

In two-thirds of the fields surveyed in 2020, the first SM captures were made during the first and the second week of June, which was a little earlier than in 2019.

Because of the high number of sites followed for SM, only the population dynamics of the nine sites followed for parasitoid emergence are presented. It is worth mentioning that an average of 20 SM/trap/day was observed at the most sensitive stage of canola. As observed in AT and BSL, two peaks of population were recorded in the site of St-Raymond (CN). The first one occurred on June 1<sup>st</sup> and the second one on June 18<sup>th</sup>, just before the elongation started. SM population remained below the presumed action threshold of 5 SM/trap/day for the rest of the season. Populations of SM in CA were the lowest of all the fields. By the time the peak happened, canola was at the flowering stage, a less sensitive stage to SM infestation. Finally, in the SLSJ region, population of SM were as high as 50 SM/trap/day and two peaks were observed for both municipalities.





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No significant correlation was found between the number of SM/trap/day and the percentage of plants showing symptoms of infestation. However, a positive trend was observed during the second period (between bolting and ripening) for the traps located in the border of the fields. The same trend was observed in 2019. The more SM were caught, the more plants were showing SM damage. The two next years' evaluations will be important to be able to draw any conclusions.

Canola plants were collected in 12 fields across seven regions of Québec in 2020: two in Abitibi-Témiscamingue (AT), two in Bas-Saint-Laurent (BSL), one in Capitale-Nationale (CN), two in Chaudière-Appalaches (CA), two in Saguenay-Lac-Saint-Jean (SLSJ), two in Gaspésie (GAS), and one in Montérégie-Est (ME). All fields were sampled four times, except for two fields which were sampled only three times. On each sampling date, 20 SM-infested plants were collected, and the infested parts were trimmed and placed in plastic emergence

containers. Samples were sent to CÉROM where they were kept under controlled conditions and checked every 1–2 days for about 6-7 weeks. SM and parasitoids emerging in the containers were collected. All SM that emerged during the first three days and three quarters of the *S. myles* from each site were kept for further identification. The remaining insects were added to the SM and *S. myles* colonies, respectively. Parasitoid occurrence and parasitism rates were determined for each site. Specimens of SM and parasitoids are then sent to AAFC researchers in Saskatchewan and the Canadian National collection for confirmation of identification. All collected samples were also checked for other potential parasitoids of Cecidomyiidae. If such insects were found, they were preserved and sent to taxonomic experts to assist with identification of all parasitoid specimens.

Both SM and *S. myles* were found in almost all regions followed in 2020 except for CN where no *S. myles* was found. Overall, three sites in CA, CN and GAS were not hosting *S. myles*. Interestingly, no *S. myles* was found for two consecutive years in the same site of CA (Saint-Lambert-de-Lauzon). In CN, even though nine *S. myles* were collected in Saint-Raymond in 2019, none were found in 2020. Together, these observations show that *S. myles* in not well established in the region where canola is not widely cultivated (CA, CN, GAS).

Of the 920 canola plants collected during the season, 365 turned out to be infested with SM (40%). From these infested plants, a total of 6 845 SM and 1 264 *S. myles* emerged from collected samples and the proportion of SM-infested plants showing parasitism was 0.36, which is lower than 2019 (0.61). The average number of SM and *S. myles* per plant was 18.8 and 3.5, respectively.

With respect to abundance and parasitism rate, significant differences were observed between regions. One fourth of the SM (1 835) and 81 % of the *S. myles* (1 022) were from plants collected in AT. The region of SLSJ was the second to host the most *S. myles*, followed by BSL (137 and 81, respectively). Very few individuals of *S. myles* were collected from sites located in CA and GAS, though the number of SM was important (344 and 695, respectively). These results are like those of 2019, where most of the parasitoids and SM were found in AT, and BSL and SLSJ were having many of the remaining parasitoids.

The global parasitism rate was 15.6 %, which is a little bit higher than the one from 2019 (12.7%). Parasitism rates were the highest in AT with 42.3 and 33.9 %. In SLSJ and BSL, parasitism rates were lower (ranging from 13.4 % to 21.5 %). In the other regions, the parasitism rates were lower than 5 %.

In 2019, some of the parasitoids that emerged from the infested canola plants were suspected to belong to the genus *Inostemma* (*Platygastridae*). These specimens were sent to the Zoological Museum, University of Copenhagen, Denmark for identification in 2020. Identification confirmed our suspicions for this genus and around 10 specimens of *Inostemma* emerged from the collected samples. We believe this is the first detection in Quebec of that genus from canola fields.

In 2020, none of the parasitoids that emerged from the infested canola plants were suspected to belong to another genus.

#### Parasitoid rearing

The *S. myles* rearing was initiated in August 2019 at the CÉROM entomology laboratory from field-collected specimens and was maintained since then. With the objective to increase the number of parasitoids produced,

an experiment was conducted over an entire generation of *S. myles*. While maintaining the colony, four egglaying sites were offered in the same cage to determine the best egg-laying site for parasitoids. Egg laying site consisted of plants at different time after SM-infestation (three and seven days) either placed as a whole or trimmed and placed in a container filled with 1-2 cm of moistened potting mix. SM and parasitoids emergences were followed daily until no emergence occurred for 14 consecutive days. Parasitism rates was calculated for each egg-laying site.

With the parasitoids that emerged from gathered samples in 2019, a laboratory colony of *S. myles* was initiated that year and kept since then in a mass-rearing set-up. At the time of this report, 29 generations of parasitoids were achieved. This rearing will be used to conduct experiment to understand the biology of *S. myles* and be able to ramp up the number of parasitoids produced. These experiments will set the base for conducting field cage experiments with different densities of swede midge and parasitoids to assess and determine the (i) time of release, (ii) efficacy of the parasitoid with different densities of swede midge, (iii) the effect of using parasitoids on the reduction of damages and (iv) the indirect effect of using parasitoids on yield. These studies will help to determine the optimal protocols for biological control releases (time of release, number of release).

A significant interaction between the number of days after SM-infestation and the egg laying site was found in the number of total emergences. A higher number of insects emerged from the containers infested by SM six days before while more emergences were observed for the whole plants infested three days before. No significant difference was found between the whole plants and the containers when infested by SM three days before.



Figure 6. Total emergence (swede midge and *Synopeas myles*) from whole plant or trimmed plant placed in a container previously infested (3- or 6-days prior being placed in the parasitoid rearing) by SM.

As found for total emergences, a significant interaction between the number of days after SM-infestation and the egg laying site was found in the number of *S. myles* emergences (Figure 7). The number of *S. myles* produced was significantly higher from the containers infested by SM six days before and from the whole plants infested three days before.



**Figure 7.** Emergence of *Synopeas myles* from whole plant or trimmed plant placed in a container previously infested (3- or 6-days prior being placed in the parasitoid rearing) by SM.

A significant interaction between the number of days after SM-infestation and the egg laying site was found in the parasitism rate (Figure 8). Parasitism rate was higher in plants than in containers. Also, the parasitism rate was higher in plants infested six days before compared to those infested three days before. These high numbers of parasitim rate can be partly link to the lower number of insects that emerged from the plants. It is also possible that the SM larvae are more easily accessible by the parasitoids to lay their eggs.



Figure 8. Parasitism rate from whole plant or trimmed plant placed in a container previously infested (3- or 6-days prior being placed in the parasitoid rearing) by SM.

Overall, these results show that a plant infested by SM three days before or a plant infested by SM six days before but trimmed into pieces and placed in a container are the best egg laying sites to produce a high number of parasitoids. This confirm that the method used at CEROM for parasitoid rearing is optimal to produce parasitoids.

#### Ontario

In the summer of 2020, SM was monitored with pheromone traps at 5 canola-growing sites in southern Ontario, four in Dufferin County (DU 1-4) near Shelburne and one in Wellington County at the University of Guelph Elora Research Station. 4 traps were placed in each and were checked twice weekly until every trap at a field caught zero SM for 2 consecutive weeks. Corresponding damage ratings on the canola at each site were not performed in 2020 because of COVID-19 restrictions.

The mean number of SM caught per trap per day was between 1.06 and 2.80. Interestingly, the mean number of SM per trap per day collected in 2019 from the Shelburne area was between 19.37 and 24.37, indicating a greater than 8-fold decrease in SM population in one year. Survey results from next year will indicate whether this reflects a downward trend of SM in that region of southern Ontario or an indication of strong population cycles over several years. High numbers of *S. myles* were collected at these same sites in 2020, which could indicate that high levels of parasitization were at least partially responsible for the low numbers of SM captured on traps. The first SM captures occurred within 1 week (June 02 – 09) of trap deployment at all 5 trapping sites, which is earlier than in 2019 (June 11 – June 18). Trap captures ended over a 3-week period (September 25 – October 15), with one site finishing 2 weeks later than the other sites, likely because it was planted 2 weeks later than all other sites.

At all 4 DU sites, there were 2 SM emergence peaks: the first in late June/early July, and the second in mid/late July (Figure 5). A small third peak is visible at DU3, possibly because that site was planted late and provided suitable hosts later into the season: there were flowering canola plants up until the field was cut, unlike the other fields, which stopped flowering much earlier. Site WE1 had 3 emergence peaks, one in late July, a second in early August, and a final peak in early September (Figure 5).



#### Trap collection date

**Figure 5.** Population dynamics of swede midge captured in pheromone traps in 4 canola fields in Dufferin (DU1 – DU4) and one field in Wellington (WE-1) Counties in 2020.

In total, twelve sites in Ontario were surveyed for *S. myles*. Five sites were surveyed weekly from June – September. These were the same sites in Dufferin and Wellington Counties where SM was monitored for. The other 7 sites had never been surveyed for S. myles and included 3 sites in Wellington County near Arthur that were surveyed one to three times; and three sites in Dufferin Country near Shelburne and one at the University of Guelph Ontario Crops Research Centre – Winchester in North Dundas that were sampled once in July.

At each sampling event, between 50 and 100 SM-infested canola plants were collected. A total of 4,050 plants were collected at Dufferin and Wellington Counties sites. Sampled plants were returned to the University of Guelph and trimmed down to their active growth points, which were then placed in emergence containers. Samples were monitored for 5 weeks for parasitoid emergence. The number of emerged S. myles was counted for every survey site, but the number of emerged SM were counted only for the 7 new sites because of COVID-19-related limitations. Parasitism rates were calculated for the new survey sites and all collected samples were checked for species of parasitoids other than *S. myles*, but none were observed. Samples were also checked for the presence of *Contarinia brassicola* flower galls and none were observed.

As SM were not counted from samples collected at the weekly sites in Dufferin County, it is difficult to compare global parasitism rates between 2019 and 2020. However, almost 3-fold more parasitoids were collected in 2020 than 2019. Most of the parasitoids were collected in Dufferin County. Therefore, in combination with the 8-fold decrease in SM trap counts from 2019, there is a strong indication for an increase in parasitism rates in Dufferin County. Interestingly, no *S. myles* were collected from the Dufferin County sites that were sampled once in 2020, despite the high numbers of *S. myles* collected from the other sites in that area. In 2020, *S. myles* were collected in every sampling location in Wellington County, while in 2019 none were collected. In 2020, none of the parasitoids that emerged from the field samples were suspected to belong to a different taxon.

#### Parasitoid rearing

A colony of *S. myles* was successfully initiated at the University of Guelph in August 2020 using adults collected from field survey samples. Three times a week, 4 cauliflower plants infested with 7–9 day-old SM larvae were placed for 2–3 days to allow *S. myles* to oviposit. Emergence of SM and *S. myles* was then monitored for 5 weeks. The emergence of *S. myles* from plants that had been infested with SM 7, 9, or 10 days before putting them in the *S. myles* colony was compared in the same colony cycle described above. Two cauliflower plants of infested with SM of each age cohort were placed in the cage with adult *S. myles* and left for 2–3 days. After parasitization, all 4 plants were removed, cut, and placed in emergence containers. Parasitism rate was calculated for every emergence container in the colony starting when the colony was initiated to assess parasitism rate over generations.

In the colony, more parasitoids emerged from plants that had been infested with SM 7 days before exposing them to *S. myles* compared to plants that had been infested with 9 or 10 days before, showing a positive correlation with younger SM larvae. These results are consistent with those found in Quebec, where 6 d old SM larvae produced the most *S. myles*, also around 45 individuals per plant.

The mean parasitism rate for the colony maintained at the University of Guelph is approximately 40%. However, the rate fluctuates, and during peaks, the parasitism rate is consistently over 70%.

#### Insecticide toxicity to S. myles

The toxicity of lambda-cyhalothrin, chlorantraniliprole, and spirotetramat to *S. myles* adults was assessed via direct and residual exposure. Lambda-cyhalothrin and chlorantraniliprole are registered for SM in canola, and spirotetramat is registered for use in cole crops where *S. myles* also may be present. The parasitoids for these experiments were obtained from canola samples collected during field surveys.

To assess the effect of topical application to adults adult *S. myles* were anesthetized and sprayed insecticide solution. Each insecticide was applied at half, full, and double its recommended application rate for SM. Mortality was assessed after 24 h. Each insecticide was also applied at half, full, or double the recommended rate to a piece of filter paper in a Petri dish to determine adult SM exposure to insecticide residues. Adult *S. myles* were anesthetized and placed on the treated filter paper in the dishes. Mortality was assessed after 24 h. Further experiments were performed with lambda-cyhalothrin to assess the toxicity of aged residues. Using the same methods as described above, *S. myles* adults were exposed to full, half, and quarter rates of lambda-cyhalothrin residues that had been aged for 1, 3, 5, or 7 d.

Chlorantraniliprole and spirotetramat were harmless to *S. myles* adults when applied as direct sprays, whereas lambda-cyhalothrin caused 100% mortality at every application rate. Similarly, chlorantraniliprole and spirotetromat as 24-h-old residues were harmless to *S. myles* adults.





In contrast, exposure to residues of lambda-cyhalothrin resulted in 100% morality of *S. myles* at nearly every rate and residue age tested (Figures 12 and 13).



Figure 13. Percent mortality (±SE) of adult *S. myles* exposed to lambda-cyhalothrin residues aged 1, 3, 5 and 7 days at full, half, and quarter rates.

Based on these results, chlorantraniliprole is non-toxic and lambda-cyhalothrin is toxic to *S. myles* adults at rates used for SM management. These data suggest that chlorantraniliprole would be preferable to lambda-cyhalothrin for an IPM system for SM management in canola designed to promote the establishment of *S. myles*; however, these results need to be confirmed in semi-field or field tests under more realistic exposure scenarios.

#### Effect of sweet alyssum on parasitism by S. myles

A lab experiment was conducted to determine if flowering sweet alyssum increases the realized fecundity of *S. myles* as part of a larger effort to assess if sweet alyssum may be a suitable supplement plant as part of a conservation biological control strategy to support *S. myles* and increase the parasitism of SM in canola. Prior to the experiment, potted canola plants were infested with SM larvae. The plants were infested prior to the start of the experiment to ensure *S. myles* had access to all larval instars of SM for parasitization. Adult *S. myles* were placed in a cage with 2 infested canola plants or a cage containing 2 SM-infested canola plants and 4 flowering sweet alyssum plants. After 6 days, the canola plants were removed from the cages, and the parasitoids were allowed to develop. The number of parasitized SM larvae and the number of *S. myles* eggs and larvae inside SM larvae per cage were then counted under a microscope.

The number of SM larvae on canola plants that were parasitized by *S. myles* was significantly higher in cages that contained flowering sweet alyssum. Similarly, the percent of *S. myles* that was in the egg stage of development was higher in cages that contained sweet alyssum plants and canola plants compared to cages with only canola, although this difference was not significant.

These results suggest that sweet alyssum may directly increase the parasitism of SM by *S. myles*. Furthermore, the higher proportion of *S. myles* in the egg stage of development in the canola + sweet alyssum treatment could suggest that sweet alyssum increases the longevity, and in turn the ovipositional period, of *S. myles*. Therefore, supplemental planting with sweet alyssum in canola may be an effective

conservation biological control strategy to support natural populations of *S. myles,* and in turn, increase the parasitism of SM.

The parasitoid *S. myles* was found in all the Quebec regions sampled in 2019 and 2020. *S. myles* was found in every county in Ontario that SM was found in. The large increase in *S. myles* numbers and decline in SM trap counts from 2019 to 2020 in the core survey region of Dufferin County indicates that *S. myles* may becoming more established in this heavy canola growing region. The new detections of *S. myles* in every Wellington County site in 2020 indicates its range is expanding. Moreover, almost 75% of the infested plants sampled in Abitibi-Témiscamingue and Saguenay-Lac-Saint-Jean also hosted *S. myles*, which suggests that *S. myles* is well established in those regions. It will be interesting to follow how the population of *S. myles* will evolve based on the acreage of canola and cruciferous crops in the different regions of Quebec and Ontario.

Finally, the rearing of *S. myles* seems to be stable and steady. The protocol was optimized in 2020 to maximize the production of parasitoids. Further refinement and laboratory trials over the next several years will make them even more efficient and will help to further develop the methods to facilitate mass rearing of *S. myles*. Laboratory and field experiments will be conducted during the two-last years of the project.

#### 2021 Progress:

Field trials are being completed as planned in both Quebec and Ontario.

### Investigation of Critical Tissue Sulfur Concentration and Nitrogen to Sulfur Ratio for Optimizing Canola Production in Eastern Canada

#### Principal Investigator: Bao-Luo Ma, AAFC, Ottawa Research and Development Centre

The best way to improve N fertilizer use efficiency (NUE) in canola is to adopt a nutrient balance approach, which considers the synergistic and antagonistic interactions between macro- and micro-nutrients that occur in soils and plants (*Fageria*, 2001). If crop growth is limited by an excess or deficiency of another essential nutrient, focusing on the crop response to N fertilizer inputs alone is unlikely to improve N fertilizer use efficiency (*Subedi* and *Ma*, 2009). Hidden hunger for micronutrients (*Gao* and *Ma*, 2015) and unbalanced nutrient supply (*Ma* et al., 2017) are the main causes of low N fertilizer use efficiency. Canola is expected to be sensitive to S concentrations in plant tissue, as S is an essential component of the amino acids cystine, cysteine, and methionine, and oilseed crops in the Brassiceae family require larger amounts of S than small grain cereal crops (*Nuttall* et al., 1987; *Grant* and *Baily*, 1993). For example, canola seed has a narrow N:S ratio of 6:1, compared to 16:1 in harvested wheat grain. On the other side, soil S source has been dramatically

reduced in the past decades likely due to (i) reduction from atmospheric deposition with the effective control of air pollution, (ii) reduction in S content in phosphorus (P) fertilizers; and (iii) wide growth of high S demand crops and/or larger S removal with increasing crop yields (IPNI, 2016). Currently, there is a lack of knowledge on the appropriate S concentrations at early growth stages to be used as a diagnosis tool for S deficiency correction and N:S ratios at maturity to assess a balanced strategy of N and S nutrition being met. The intention of this study is to address these issues.

Experimentation is being conducted at two locations in Eastern Ontario: The Central Experimental Farm in Ottawa, ON and MacDonald College of McGill University. At both locations, the experiment was planted in two fields with different soil characteristics. Ottawa sites are sandy clay loam and sandy clay, while MacDonald College sites are CHICOT sandy loam and Rosalie clay.

#### **Results to Date:**

In both 2019 and 2020, planting was delayed. 2019 due to extremely wet spring and 2020 because of COVID restrictions.

In 2019, it was an extremely wet April and May with cooler than normal temperatures. From May 1-May 30 there were only 250 heat units, where the 30-year norm is 350 heat units. In Ottawa, it was considered a state of emergency by the end of April because of all the terrible flooding. July had very little precipitation with hotter than normal temperatures. The cool wet spring made it difficult to work the land, fertilize and plant early. Planting occurred about 2 weeks later than normal. At both sites in Ottawa, compaction from the tractor tires was a problem causing plants in the tire marks to be shorter than the other plants in the plots. In July the ground was so dry, soil sampling was difficult. Ste-Anne-de-Bellevue had a similar spring and summer.

In 2020, there was extremely low precipitation and higher than normal temperatures for May, June, and July. June had 15 mm of rain, much lower than the normal 93 mm of rainfall for that month. According to Environment Canada, it was the hottest July on record since 1921. From May 21 to Sept 1, there were 31 days of above 30°C temperature when the normal is 14-15 of those days in a summer. This affected both germination and flowering. Germination was poor, flowering was short, and pods had fewer seeds. McGill also had lower than average rainfall for both May and June. But they had twice as much rain as Ottawa in July (87.6 mm). Due to COVID-19 restrictions, planting occurred about 3 weeks later than the site weather conditions. The late sowing also exacerbated the negative impact of drought on plant growth and development.

#### Phenological Progression

In 2019, plants emerged and grew normally at all sites. Flea beetles were not an issue at either location. Only in Ottawa compaction became a problem because the soil was so wet when planting occurred. Plants that were planted in the tire tracks were slower to emerge and were shorter than the other plants in the plots. Because of this, measurements were avoided in those areas.

At the Ottawa clay site in 2019, the plots with high N rates (160 and 240 kg ha<sup>-1</sup>) and no sulfur, had plants that exhibited signs of S deficiency early on in their growth. By the rosette stage, leaves were beginning to cup and the leaf edges turned purple (Figure 1A). These plants were much slower to develop than the plants in the other plots. They flowered much later, and the flowers were a very pale yellow. Many of the flowers did not produce seed pods, leaving the plants with very few pods (Figure 1B). The Ottawa clay site was the only site that exhibited these signs of S deficiency. S, unlike N, is immobile in plants and will not translocate from older to newer vegetative material. So if there is not enough soil sulfate to take up by the plant, S deficiencies would be observed in the most recently developed leaves and flowers. Excessive amounts of nitrates have also been known to reduce the uptake of available sulfate, especially when S is already deficient in the soil.

In 2020 at Ottawa, emergence was poor because of the extremely dry weather, especially at the clay site. There was no lodging at either site because the plants were short with very few pods. Flea beetles caused a lot of damage to the pods at the sand site, but not as much at the clay site (Figure 2). There was also a dry spell for May and June after planting at the McGill site. There was no lodging and some flea beetle pressure.



B)



**Figure 1.** Sulfur deficiency symptoms of the canola plants. A) the cupping and purpling of the leaves. B) how few pods formed by maturity.



Figure 2. Flea beetle damage to the pods at the sand site in Ottawa in 2020.

#### Soil Nitrate and Sulfate Levels

Preplant soil samples were taken at all sites and results. In 2020, because of COVID, the preplant samples in Ottawa were analyzed in the ORDC Chemistry lab instead of an accredited lab in Ottawa. Therefore, some parameters were not measured. Soil organic matter (%) was very low at the McGill clay site in 2020 with a value of only 2.4% (Table 1B).

Table 1 A & B.	Results of preplant soil samples (0-15 cm) taken at each site in Ottawa and Ste-Anne-de-Bellevue in the
spring in 2019	(A) and 2020 (B).

A) 2019

	Ottawa Sand	Ottawa Clay	McGill Sand	McGill Clay
Previous Crop	Corn	Corn	Soybean	Corn
Soil Type		sandy clay loam	CHICOT fine	Rosalie clay
	sandy clay		sandy loam	
Soil Texture	36% clay-49%	27% clay-51%	8.5% clay-55%	37% clay-27%
% clay, sand, silt	sand-15% silt	sand-22% silt	sand-36.5% silt	sand-36% silt
OM (%)	3.8	3.6	3.14	6.28
P (ppm)	18	36	57.82	30.19
K (ppm)	100	120	83.7	174.39
рН	5.1	5.6	7.02	7.01
Total CEC (meq/100g)	10	13	14.3	32

B)	2020
- /	

	Ottawa Sand	Ottawa Clay	McGill Sand	McGill Clay
Previous Crop	Corn	Oats	Fallow	Alfalfa
Soil Type	Sandy Loam	Clay Loam	CHICOT fine	MacDonald
			sandy loam	clay loam
Soil Texture	12% clay, 62%	35% clay, 45%	-	-
% clay, sand, silt	sand, 26% silt	sand, 20% silt		
OM (%)	3.6	2.9	2.9	2.4
P (ppm)	9.6	10.1	225 (kg/ha)	157 (kg/ha)
K (ppm)	-	-	280 (kg/ha)	238 (kg/ha)
рН	6.9	6.8	6.6	6.4
Total CEC (meq/100g)	-	-	13.9	15.7

Preplant soils were analyzed for available nitrate, ammonia, and sulfate in the laboratory at the Central Experimental Farm. The clay site in Ottawa in 2019 had the lowest preplant sulfate level of only 3.8 ppm. For all sites-years and each growth stage, soil nitrate levels significantly increased with increasing amounts of N fertilizer (Figure 5A & B).





Figure 5 A (2019) & B. Soil Nitrate levels at the 4 leaf, rosette (McGill only) and 20% flowering for the clay and sand sites of Ottawa and McGill in 2019 (A) and 2020 (B).

Of the eight sites-years, only the Ottawa and McGill sand sites in 2019 did not show significant differences in soil sulfate levels between the plots that received 0, 20 and 40 kg S/ha. For all the other sites, the 0 S plots had the lowest soil sulfate levels at each sampling, and the 40 S plots had the highest soil sulfate levels, usually significantly so.

There was no significant N x S interaction effect on soil available sulfate at any of the site-years and growth stages, except for the Ottawa clay site in 2019. Even though the N x S interaction was not significant, by 20% flowering stage, the available soil sulfate levels were much lower in the 160 N and 240 N plots with no S, compared to all the other treatments and even lower than in the plots with no N or S.

More work needs to be done to determine why this is happening at this clay site and not the other 7 sitesyears. But excessive amounts of nitrates have been known to reduce the uptake of available sulfate. It should be noted that only at the Ottawa clay site in 2019 did the addition of N have a slightly significant effect on soil available sulfur, causing it to lower with increasing applied nitrogen.

#### **Final Yields**

Yields in 2020 were much lower than those of 2019 for both sites due to the extremely dry and hot conditions in May, June, and July. The Ottawa sand site in 2020 had the lowest average yield (377 kg ha<sup>-1</sup>) of all site-years with no significant yield response to the different levels of N fertilizer. At all the other sites, yields responded positively to increasing amounts of preplant N fertilizer with the 0 N plots significantly having the lowest yields and, in most cases, (except the Ottawa clay site in 2019), the 160 and 240 N plots having the highest yields.

Of the 8 site-years, only at the Ottawa clay site in 2019 and the McGill clay site in 2020 did the application of preplant S fertilizer have a positive effect on yields, with the lowest yields in the 0 S plots and having the highest yields in the 20, 30 and 40 S treatments. There are a couple of reasons why these sites might have shown this positive yield response to increasing S fertilizer rates. The Ottawa clay site in 2019 had the lowest

preplant soil sulfate level of only 3.8 ppm compared to 7.1 ppm of all the other site-years. Because the initial soil sulfate level was very low and therefore not supplying enough sulfate to the plant, it would be reasonable to expect a crop response to additional S fertilizer application. For the McGill clay site in 2020, the soil had the lowest organic matter content of only 2.4%, compared to the average organic matter content of 3.7 % for the other 7 site-years. Soil S is in the form of organic matter. It needs to be broken down to sulfate to be available to the plants. Organic matter content below 3% may not supply enough sulfur for plant needs, especially if the conditions are not right (i.e. optimum soil temperature, adequate moisture, microorganisms, etc.). So, it is possible that the soil sulfate levels were already very limiting, and thus a positive yield response to the addition of S fertilizer was shown. For all the other sites, it is possible that the soil sulfur levels were not limiting (according to the initial soil sulfate results) due to the higher organic matter content, therefore providing enough sulfate to the plants. Consequently, the addition of S fertilizer did not show a yield response.

The Ottawa clay site was the only site that had a significant N x S interaction on yields, with the plots that received no N or S having the lowest yields and yields increasing with increasing N and S. However, the plots with high N rates (160 and 240) and no sulfur had the poorest yields, even worse than those of the 0 N plots. These are the same plots where the plants showed signs of S deficiency with purple and cupped leaves, were less green, took longer to develop, flowered later, and produced few and small seed pods. The Ottawa clay site was the only site that exhibited these signs of S deficiency. These plots also had the lowest soil sulfate levels by the 20% flowering.

#### Most Economic Rate of Sulfur (MERS) Calculations

There were no positive yield responses to S at the Ottawa sand 2019, McGill sand and clay 2019, Ottawa sand and clay 2020, and McGill sand 2020. Of the 8 site-years, only Ottawa clay 2019 and McGill clay 2020 showed a positive yield response to S fertilizer (Figure 8).



**Figure 8.** Regression analysis of grain yield as a function of sulfur fertilizer applied preplant for the two sites that showed a positive yield response to sulfur in 2019 and 2020.

If Ottawa and McGill can represent Ontario and Quebec, the average MERS value would be 28.5 kg ha<sup>-1</sup> for preplant S (Table 8). This would produce an average yield of 2550 kg ha<sup>-1</sup> with a yield increment of 63 kg for every kg S. More site-years need to be tested to get representative data.

**Table 8.** The calculated most economical rate of S (MERS) for preplant application for the site-years that showed a positive yield response to S.

Site	Year	MERS (kg N ha <sup>-1</sup> )	Estimated yield at MERS (kg ha <sup>-1</sup> )	Estimated yield increment (kg seed / kg S)	S rate (kg ha <sup>-1</sup> ) at the highest yield	Observed highest yield (kg ha <sup>-1</sup> )
Ottawa						
Clay	2019	30.4	3013	85.8	30	3010
McGill						
Clay	2020	26.6	2087	40.4	20	2100
		28.5	2550	63.1	25	2555

#### Plant Height, Branches, Pods, seeds per pod and Thousand Seed Weight

Plant heights were not measured in Ottawa in 2020 due to COVID restrictions. However, for most other siteyears, plant heights increased significantly with increasing N fertilizer levels. Sulfate fertilizer had no effect on plant height.

At all sites, the number of branches and number of pods per plant increased significantly with increasing N fertilizer rates. Number of seeds per pod, also increased with increasing N levels. In all cases, the plots that received the least amount of N had the least number of branches, pods, and seeds, and in most cases, the plots that received 240 kg ha<sup>-1</sup> preplant, had the most branches, pods, and seeds/pod.

It is difficult to determine the trend of thousand seed weight (TSW). At 3 site-years, TSW decreases significantly with increasing preplant applied N and at 3 site-years, it increases with increasing N rates.

Sulfur did not have any effect on number pods, seeds per pod or thousand seed weights at any site. However, the addition of S fertilizer did influence number of branches per plant but was not consistent. For 5 of the 8 sites-years, branching either increased or decreased significantly with increasing amounts of preplant S.

The Ottawa clay site is the only site that had a very significant N x S interaction on number of seeds per pod, number of pods  $m^{-2}$  and seeds  $m^{-2}$ .

Using the stand counts, pods/plant and seeds per pod, number of pods m<sup>-2</sup> and seeds m<sup>-2</sup> were determined. Pods m<sup>-2</sup> and seeds m<sup>-2</sup> increased significantly with increasing N fertilizer rates. Note that the number of pods in Ottawa in 2020 was extremely low compared to the other sites. This corresponds to the extremely low yields in Ottawa that year. The pod and seed m<sup>-2</sup> data from the McGill site in 2020 appears to be inconclusive as the McGill clay site has much higher pods m<sup>-2</sup> and seeds m<sup>-2</sup> counts than the McGill sand site 2020 yet the yields are lower at the McGill clay site (1908 kg ha<sup>-1</sup>) than the McGill sand site (2330 kg ha<sup>-1</sup>). The addition of sulfur did not have any effect on pods m<sup>-2</sup> for any of the site-years. Only for the Ottawa clay site in 2019 did the addition of sulfur significantly increase seeds/m<sup>2</sup>.

Based on analysis, there is very strong positive correlation between yield and plant height for 5 of the 6 sites where it was measured. There is also a very strong positive correlation between yield and the number of branches/plant for 6 of the 8 site-years and number of pods/plant for 7 of the 8 site-years. Finally, it was determined that there is a strong negative correlation between thousand seed weight and yield.

#### **Oil and Protein**

Oil and protein values are reported at 8.5% moisture. Increasing rates of preplant N significantly increased seed protein concentration (%), but decreased oil concentration. In all cases, the plots that received 240 kg N ha<sup>-1</sup> had the highest seed protein concentration but the lowest seed oil concentration. Plants from the 0 N plots had seed with the lowest protein concentration but highest oil concentration of all the treatments. The addition of sulfur fertilizer significantly increased protein and decreased oil concentrations at the Ottawa sand sites in 2019 and 2020.

Based on analysis, yield is positively correlated with protein but negatively correlated to oil for 3 of the 4 sites in 2019. It is the Ottawa clay site that does not show the correlation for either protein or oil. For 2020 only the Ottawa sites have been analyzed and their oil and protein results are opposite to 2019.

#### **Canopy Reflectance (NDVI)**

Canopy reflectance measurements determining plant greenness, were taken with the Greenseeker instrument several times during the growing season at the Ottawa location only. From the 4 leaf stage to 20% flowering, greenseeker detected significant differences in NDVI between the plants in the 0 N plots and plants receiving N treatments, with increased NDVI readings with increasing N application. Only the Ottawa Clay site in 2020 at the 4 leaf stage did not show any significant differences. This was probably due to the uneven emergence and all small plants caused by the extended period of drought and hot stress. The addition of sulfur had no effect on canopy greenness.

There is a very strong positive correlation between NDVI readings and yield based on the Pearson's coefficient analysis and regression analysis (Figure 10A) in 2019. However, at the clay site in Ottawa at 20% flowering, there was a negative correlation between yield and Greenseeker, probably because the plants in the 0 S plots with 160 and 240 kg N/ha were so far behind in development with much lower yields, compared to plants in the other treatments.

In 2020 only the Ottawa clay site showed a strong positive correlation between yield and Greenseeker at the 20% flowering stage. The Ottawa sand site showed no correlation because the yields were so low and not significantly different between N treatments.

#### Leaf Chlorophyll Readings (SPAD)

SPAD readings from the SPAD-502 leaf chlorophyll metre were measured at both the Ottawa and McGill sites. Readings were taken from the 4-leaf stage to 20% flowering. At all growth stages, SPAD could detect leaf chlorophyll differences, with SPAD readings significantly increasing with increasing preplant N rates.

In 2019, there was a very strong positive correlation between SPAD readings and final yields at the Ottawa and McGill sites based on Pearson's correlation and regression analysis. In 2020, only the McGill site showed a positive correlation between yield and SPAD readings.

#### Plant Nitrogen Accumulation (kg ha<sup>-1</sup>)

Nitrogen accumulation (kg ha<sup>-1</sup>) in the plants at the 4-leaf stage, the beginning of flowering and in the straw and seed at maturity significantly increased with increasing N fertilizer rates at all 4 sites in 2019 and 2020 with plant material at all growth stages accumulated the highest amount of N at the 240 kg N ha<sup>-1</sup> fertilizer rate. Nitrogen accumulation in the plant material also increased from the 4-leaf stage to maturity. At maturity, in 2019 nitrogen accumulation was greater in the seed than the straw material for all 4 sites. However, in 2020, at both sites in Ottawa, there was more N accumulation in the straw material than the seed.

If N accumulation in the plant at early flowering is compared with the N accumulated in the plants at maturity, it is noted that most of the N accumulated in the plants was taken up by flowering. For example, at the Ottawa sand site in 2019, by 20% flowering the plants had accumulated 135 kg ha<sup>-1</sup> of N. By maturity the plants accumulated 140 kg ha<sup>-1</sup> of total N.

At 13 of the 40 growth stage-by-site-by-year results, sulfur accumulation did increase with increasing N fertilizer rates. At the 2019 Ottawa clay site, the 0 N plots had the lowest S accumulation, but the S accumulation decreased from the 80 to 240 N plots. The 2019 McGill sand site saw a significant decrease in S accumulation with increasing Fertilizer N rates at all growth stages.

The Ottawa sand and clay sites in 2020 had the lowest nitrogen accumulation in their seed which corresponds to the lowest yields of all site-years.

#### **N:S ratio**

All crops need N and S for protein. Crops have varied requirements for S compared with N and have a wide N:S ratio in the harvested product. The optimum N:S ratio for canola plant is about 6:1 as canola has a higher requirement for sulfur than other crops.

The N:S ratios increased significantly with increasing fertilizer N rates. In this study, the final N:S ratio for seed material ranged from 8:1 (Ottawa Sand 2020) to 15:1 (Ottawa sand 2019). These ratios indicate that possibly more sulfur was needed or the possibility that too much N was added. If a crop cannot find an adequate balance of all the nutrients it needs it will not perform to its potential.

It was noted that plant N:S ratio varied largely among different environments (sites-years). It was not only affected by nutrient supply but was also dependent on the growth stages and plant tissues. The close relationship between N:S ratio and final yield indicates that there existed a critical N:S ratio, that is, the

balanced uptake of N and S, to realize the yield potential. Identification of this optimum ratio requires multisite-year tests.

#### Nitrogen remobilization quota (NRQ), and Post-flowering Nitrogen uptake (PNaU)

At all sites except the Ottawa clay site in 2020, the nitrogen remobilization quota (or the pre-flower N accumulation) (NRQ, kg ha<sup>-1</sup>) increased significantly with increasing N fertilizer rates, and the post-flowering N uptake (PNaU, kg ha<sup>-1</sup>) was decreased significantly as N fertilizer rates increased. For each site, the pre-flowering N accumulation at each N rate was greater than 0, meaning that the canola's pre-flowering nitrogen resources were sufficient to cover the requirements of the growing seed during the seed filling period.

Only in the plots that received 0 or low N rate (80 kg ha<sup>-1</sup> N fertilizer), was there post-flowering N uptake (PNaU) from the soil for the developing seed. However, no more extra N was taken up by plants post-flowering in the plots that received 160 and 240 kg N ha<sup>-1</sup>, as indicated by the negative PNaU value. Any post-flowering uptake value that is negative means that no more N was needed for the developing seed as there was a sufficient supply of pre-flower N remobilized from the plant vegetative material (leaves and stalk) to the developing seed.

The Ottawa clay site in 2020 was different. Pre-flower N accumulation decreased with increasing N fertilizer and post-flowering N uptake increased with increasing N fertilizer application. At N fertilizer rates of 160 and 240 kg ha<sup>-1</sup> there was not enough pre-flowering N accumulation in the vegetative material to support seed development (negative values) so most of the N for seed development was taken up post-flowering from the soil. Severe drought and hot stress during the vegetative growth stage, limiting canopy photosynthesis, may not provide sufficient carbohydrates for root growth. This, in turn, the reduced root size and may have restricted the root area exploration and thus resulting in the reduced pre-flowering N accumulation in the vegetative tissues. When drought was alleviated, the crop may invest more energy to the roots and stimulate re-initiate branching, leading to non-productive regrowth (Fig. 13), lower harvest index and reduced yields. This requires further investigation.



**Figure 13.** A photo showing the regrowth of canola plants during the pod filling stage once the drought stress has been released after rain.

#### Plant Sulfur Accumulation (kg ha<sup>-1</sup>)

In 2019 and 2020, sulfur accumulation (kg ha<sup>-1</sup>) in the plants increased significantly with increasing sulfate fertilizer rates. Plants fertilized at 40 kg S ha<sup>-1</sup> accumulating the highest amount of S. For both sites at McGill in 2019, S fertilizer rates did not have a significant effect on S accumulation in the plant material.

The accumulation of S in the plants increased from the 4-leaf stage to 20% flowering. In most cases, there was more S accumulated in the straw material than in the seed, therefore the N:S ratio of the straw material was often smaller than that of the seed. This indicates that at the post-flowering stage, the rate of S remobilization from the vegetative to seed is much smaller than that of N remobilization. At most sites, at least half of the S in the plants at maturity was accumulated by flowering.

#### Sulfur remobilization quota (SRQ), and Post-flowering sulfur uptake (PSaU)

The pre-flowering S remobilization quota (SRQ) and post-flowering S uptake were very different for each siteyear. At the Ottawa sand site in 2019, pre-flowering S accumulation (SRQ) increased significantly with increasing sulfur fertilizer rates. The S accumulation at each rate was greater than 0, meaning that the canola's pre-flowering sulfur resources in the plant vegetative tissues were sufficient to cover the requirements of the growing seed during the seed filling period. No post-flowering S uptake by the plants was required at any of the S fertility levels as indicated by the negative S uptake values. At the Ottawa clay site in 2019, the pre-flowering S remobilization quota was negative in the plots with zero S added, requiring the plants to remove more sulfur from the soil for seed development. In the plots that received sulfate fertilizer, enough S accumulated in the vegetative material for seed development (positive SRQ values) that very little S was need from the soil during the seed filling period. In fact, in the 40 kg S ha<sup>-1</sup> plots, no more S was taken from the soil post-flowering. At the site in 2020, S accumulation (SRQ) in the vegetative material also increased with S fertilizer levels, however, the pre-flowering S accumulation in the vegetative material of the plants in the plots that received no S fertilizer was not sufficient to cover the requirements of the growing seed during the seed filling period. This forced the plants to take up S from the soil during this period. The plants in these same plots took up more S post-flowering from the soil for seed development. In the plots that received 40 kg S ha<sup>-1</sup>, no extra S was needed by the plants post-flowering during the seed filling period.

For both McGill sites in 2019 and the Ottawa sites in 2020, the pre-flowering S accumulation values were all negative at each S fertilizer level, with the plots that received 40 kg S ha<sup>-1</sup> having the most negative value. This indicates that the pre-flowering S accumulation in the vegetative material was not sufficient for the growing seed and therefore the plants had to take it up from the soil. More S was taken up by the plants post - flowering with the highest S uptake in the 30 and 40 kg S ha<sup>-1</sup> plots. There are several reasons for the negative S remobilization quotas. Weather conditions may not have made sulfate available to the plants, due to leaching (as in 2019) or extremely warm temperatures and the lack of rain (in 2020).

#### **Correlation of Nutrient Accumulation with Yield and NDVI**

Plant total N at flowering, straw, seed, and plant total N at maturity were all positively correlated to yield. There is also a strong positive correlation between the nitrogen remobilization quota and yield at some of the sites. There is no correlation with yield and post-flowering nitrogen uptake.

Total sulfur accumulation in seed and plants at maturity both have a strong positive correlation with yield. In general, there did not seem to be any correlation with yield and pre-flowering S accumulation or post-flowering S Uptake.

There is a strong positive correlation between NDVI and total plant nitrogen (kg ha<sup>-1</sup>) from the 4-leaf stage to 20% flowering. This is also true for SPAD, however the strong positive correlation often began later at the rosette stage.

There is not a consistent significant positive or negative correlation between SPAD or NDVI measurements and plant total sulfur accumulation (kg ha<sup>-1</sup>).

#### 2021 Progress:



Although severe droughts occurred during most of the vegetative growth period, the 2021 canola field trials (pictured) look much better than 2020 and good yields are expected.

### Environmental and Economic Impact of Canola in Potato Rotation in Eastern Canada

#### Principal Investigator: Aaron Mills, AAFC-Charlottetown

The previous ECODA cropping systems project evaluated 10 different rotations that were approved by industry collaborators. These rotations were conducted over a period of three years and provided information regarding the effects of the presence of canola, soybean, and corn, in a rotation with potatoes. The project also generated information on the influence of crop diversity within each of the cropping systems. The previous project work showed that sequential planting of soybean and canola showed the highest percentage of culls in the potato crop of all rotations. The highest overall potato yields were observed in the corn/canola rotation. We collected limited information on soil nutrient and soil health status including nematode population dynamics and phospholipid fatty acid profiles. These measurements showed that higher mycorrhizae numbers were observed in rotations containing forage, forage mixes and grains; plant parasitic nematodes showed slightly higher numbers in the corn-canola rotation and were lowest in the corn-soybean rotation when averaged over the three years. When broken down by year, certain rotations resulted in an increase in plant parasitic nematode numbers such as canola-canola, barley-forage, and canola-wheat; however, these numbers did not appear to correlate negatively with potato yield.

The current cropping system study is evaluating different management practices that can improve both the sustainability and profitability of potato rotations with the following objectives:

- 1. Agronomy: cover crop compatibility for each cropping system; effects of fall plow vs. winter cover crop; yield components and agronomic metrics of all crops in all rotations; disease effects on all crops including potato
- 2. Nutrient management : N credits from different cover crops ; N use efficiency ; Soil enzyme activities associated with C, N, and P cycling
- 3. Soil health: nutrient ratio in the whole soil and microbial biomass; soil aggregate stability and particulate organic matter during potato phase; effects of brassicas on mycorrhizal colonization of subsequent crops; evaluate mycorrhizal inoculant application to improve potato yield and quality

This cropping system study strategy applied the use of overseeding and under seeding to provide winter cover crops to reduce erosion and nitrogen leaching through the winter. Therefore, as a split within the design, one half of each plot was plowed in the fall to leave the soil exposed; the other half of the field was plowed in the spring. With the project being a continuation of the previous funding cycle, 2017 and 2018 were the first and the second year of the three-year potato rotation. The 2019 growing season was the potato phase while 2020 returned to an evaluation of rotational crops (Figure 1).

	2017		20	18	2019	2020		2021		2022
Rotaton	Main crop	Cover crop	Main crop	Cover crop	Main crop	Main crop	Cover crop	Main crop	Cover crop	Main crop
1	barley	red clover u/s	red clover	red clover	potato	barley	red clover u/s	red clover	red clover	potato
2	canola/pea	winter wheat	winter wheat	red clover	potato	canola/pea	winter wheat	winter wheat	red clover	potato
3	soybean	ryegrass o/s	corn	ryegrass o/s	potato	soybean	ryegrass o/s	corn	ryegrass o/s	potato
4	canola	cereal rye	реа	cereal rye	potato	canola	cereal rye	pea	cereal rye	potato
5	canola	red clover	corn	ryegrass o/s	potato	canola	red clover	corn	ryegrass o/s	potato
6	soybean	mustard	corn	ryegrass o/s	potato	soybean	mustard	corn	ryegrass o/s	potato
7	реа	cereal rye	canola	cereal rye	potato	реа	cereal rye	canola	cereal rye	potato
8	corn	ryegrass o/s	canola	cereal rye	potato	corn	ryegrass o/s	canola	cereal rye	potato
9	canola	winter wheat	winter wheat	red clover o/s	potato	canola	winter wheat	winter wheat	red clover o/s	potato
10	реа	winter wheat	winter wheat	red clover o/s	potato	реа	winter wheat	winter wheat	red clover o/s	potato

#### o/s = overseeded u/s = underseeded

Figure 1: Cropping systems implemented in the present study.

In 2020 this project fell under Phase 1 activities for AAFC COVID-19 access restrictions. As a result, the research team was able to get this experiment planted for the 2020 growing season. Due to challenges with the establishment and survival of corn in earlier years, corn plots were replaced with other field crops in rotation. The planting was slightly delayed, as most of the prep work that normally would have been done in April, was pushed back due to laboratory access restrictions. In some cases, the seeds were being weighed in the field immediately before planting. We were able to continue with the regular soil and tissue sampling; however, biomass sampling, mycorrhizae and nutrient dynamic soil sampling did not occur. The backlog of 2019 nematode community and PLFA soil sample processing is currently under way concurrently with the 2020 samples. Fall plowing occurred in all plots, rather than just added as a split; this will be dealt with when the data are analyzed globally at the end of the project. As 2020 was a drought year with extremely dry conditions for most of July, yields are anticipated to be quite low relative to previous years of this activity.

#### **Results to Date:**

#### 1<sup>st</sup> Phase Completion

End of first phase was marked by the completion of the 2019 potato crop year. All data was collected from the potato crop year, however, only the specific gravities and wireworm assessment to be done. Agronomic data is currently being analyzed with the output of the analysis used for cost-benefit analysis.

#### 2<sup>nd</sup> Phase Initiation

2020 marked the initiation of the second set of 3-year rotations at the AAFC PEI site. Despite a late start and significant challenges due to COVID-19 restrictions all plots were seeded, harvested, and data collected. Later field access due to COVID-19 restrictions resulted in slightly lower crop vigor and potentially lower yields for this year. Data will be dealt with accordingly when performing the global analysis. All yields should be lower for 2020 because of the later planting.

Fall plowing split was not performed in all plots as the first phase of the experiment clearly showed that there was a yield advantage to fall vs. spring plowing before the potato crop. Although the yields were higher, this is not the most sustainable option. The "plow" split in the research design will be maintained for the duration of the experiment to measure the legacy effects of previous fall plowing events on subsequent crops.

#### 2021 Progress:

2021 field trials being conducted as planned, with establishment of second set of rotational crops followed by the final potato crop in 2022.

### **SOYBEAN REPORT**

There are 5 soybean research activities being conducted under the current ECODA CAP program in collaboration with Sevita Genetics. Three of these projects are being conducted by Sevita researchers aimed at developing commercially viable, IP, non-GM soy varieties. The remaining 2 projects are being conducted by AAFC researchers Malcolm Morrison and Elroy Cober out of the Ottawa research station. Please note that in order to protect the IP that is being created data is not being published.

## Targeted gene stacking to create a high yielding soybean with high value, high oleic, low linolenic, low palmitic oil composition

#### Principal Investigator: Sevita Genetics / Sevita International

The development of a soybean line that contains a high oleic, low linolenic and low palmitic acid profile will yield results that will help the Canadian soybean industry grow economically by diversifying products for the global market. These specialized soybeans will further benefit Canada and the international community with the stacking of designer traits to help minimize environmental impact by reducing inputs. Additionally, the development of soybean oil used in industrial systems will help alleviate the depletion of fossil fuels across the world. Providing healthy, safe food for the world's population is a priority for all nations. A non-transgenic soybean that can provide similar yields to transgenic plants as well as enhanced traits customized to the desired end user increases public trust in Canadian soybean products. As regulation and tariffs across the globe are becoming more restrictive, the innovations of Sevita International and ECODA specializing in non-GMO soybeans will give Canada a competitive advantage on the world market.

# Mine the Mutant population for a line with high methionine and lysine

#### Principal Investigator: Sevita Genetics / Sevita International

Soybeans are a source of both Met and Lys and one of the most predominant sources of a complete protein in the world for both human and livestock consumption (Mariashibu et al. 2013). Identifying and developing a soybean that has elevated levels of these amino acids would be beneficial economically and help address some humanitarian concerns (Galili and Amir. 2013). Based on the 2016 report from the Canadian Field Crop Research Alliance finding a soybean line with increased quantity and quality of protein is one of the top 10 research priorities.

To date combinations of conventional, mutational breeding, genetic engineering, marker assisted selection and genomic analysis have been utilized to improve the protein quality and composition of grains, including soybeans (Wenefride, I et al 2013). Sevita International in association with ECODA is working toward the mining of the existing mutants and techniques developed previously to identify and develop a line of nongenetically modified (non-GMO) soybeans with high Met and Lys levels. Through the development of advanced technologies and innovative approach, Canadian growers will eventually have an additional non-GMO soybean that has enhanced nutritive quality. This will open new markets (domestic and international) and stimulate economic growth for the whole value chain. Diversifying varieties grown will also help mitigate both agronomic and economic risks for producers.

Galili G and Amir R.2013. Fortifying plants with essential amino acids lysine and methionine to improve nutritional quality. Plant Biotechnol J, 11:211-222 doi:10.1111/pbi-12025

Mariashibu T.S, Anbazhagan V.R, Jiang S, Ganapathi A and Ramachandran S. 2013. In vitro Regeneration and Genetic Transformation of Soybean: Current Status and Future Prospects, A Comprehensive Survey of International Soybean Research - Genetics, Physiology, Agronomy and Nitrogen Relationships, Prof. James Board (Ed.), InTech, DOI: 10.5772/54268.

Wenefrida I, Utomo HS, and Linscombe SD .2013. Mutational breeding and genetic engineering in the development of high grain protein content. J Agric Food Chem. 2013. 61(48):11702-10. doi: 10.1021/jf4016812.

### Targeted gene stacking to create a productive high protein soybean line for aquaculture feed

Aquaculture is a multi-billion-dollar industry in Canada, Atlantic Canada contributing over 50% of Canada's total aquaculture production. Like any animal production system, feed is the largest operating cost for in any aquaculture production system. Fishmeal has been the traditional protein and fat source for aquaculture systems but has been incrementally replaced in the aquaculture diet due to exorbitant prices and lack of supply. To decrease production costs aquaculture feed producers are replacing fish meal and oil with plantbased products, many using soybean meal as a source of high-quality protein and oil (Newkirk, 2010). Soybean meal offers a high-quality renewable protein source and can help replace at least one half to a third of the fishmeal used in the feed ration, reducing the pressure on world wild caught fish stocks (United Soybean Board, 2017).

At the current time, salmon feed that is produced and sold in the EU must contain all non-GMO ingredients. Finding a good quality source of non-GMO Soybean Meal (SBM) and Soy Protein Concentrate (SPC) can be challenging, and most is imported from ingredient suppliers in Brazil. Having a reliable source of high-quality non-GMO SBM and SBC would be very attractive for the market.

The primary anti nutritional factors that are innate to soybeans and limit their use in aquafeed diets are lipoxygenase, stachyose, phytate, trypsin and saponins. There is an opportunity to address these specific limitations of soybean feed products by using non-GMO breading innovations. The objective of this research is to incorporate high protein, amino acid and minimize antinutritional composition for the aquaculture feed market.

Newkirk, R.2010. Soybean: Feed Industry Guide 1<sup>st</sup> ed. Canadian International Grains Institute. Pp 36-39 <u>https://cigi.ca/wp-content/uploads/2011/12/2010-soybean-feed-industry-guide.pdf</u> United Soybean Board.2018. Approval of aquaculture feed could increase demand for soybean meal. <u>https://unitedsoybean.org/article/approval-of-</u>aqua-feed-could-increase-demand-soybean-meal accessed: February 2018

# Screening for soybean varieties for moisture stress tolerance and prolonged N2-fixation under moisture stress

Moisture stress is the major abiotic constraint to high stable yields in Eastern Canada (Sinclair et al. 2007). In the 16 years since the turn of the century the region has had 6 summers that were dryer than the long-term average. That is one year in three with moisture stress. Soybean yield is reduced by moisture stress during sensitive periods like flowering (Morrison et al. 2006). Plant breeders can't rely on dry conditions yearly, so a method that identifies lines with high yield in dry and in normal conditions is needed. One method is to supply the field grown plants with water every day and then compare their yield to the same plants growing near-by that only receive natural rain (Lakshmi et al. 2009). This is called the Delta Yield concept because it is based on the difference between the yield of the well-watered plants and the natural-watered ones. The cultivar with the lowest Delta Yield is the most moisture stress-tolerant yet won't suffer a yield drag when there is no drought. This method has been used in the USA to develop a water use efficient corn hybrid that yields 7.4 % higher in moisture stress and 3.4 % higher in normal water situations. Lakshmi P. et al. 2009.Plant Cell Physiol. 50:1260-1276

Morrison M.J. et al. 2006. Can. J. Plant Sci. 86:1327-1331 Sinclair T.R. et al. 2007. Field Crops Res. 101:68-71.

# Incorporation of strong seed coat, good germination, and root quality in food-grade soybeans

Germination and fermentation processes of soybeans provide health benefits because of the protein, increased levels of phytosterols and tocopherols (Shi et al 2010). Therefore, in addition to the swelling population in soy-loving Asian countries, the increased focus on healthy and sustainable eating and the fusion of cultures across the globe, soy-food such as natto and soybean sprouts are increasing in popularity. Due to the increase in demand for food grade soybeans it is essential to improve soybean cultivars that can efficiently produce high quality soy-food products (Murugkar, 2014).

The objective of this work is to develop small-seeded soybeans for specialty markets, namely natto in Japan and sprouting soybean in Korea. Both markets require soaked seeds as part of production and water uptake can play a role in product quality. Seed coat parameters will be investigated as part of the soaking process.

Murugkar, D.A. 2014. Effect of sprouting of soybean on chemical composition and quality of soymilk and tofu. J.Food Sci Technol 51(5):915-921. Shi H., Nam P.K., Ma Y. 2010. Comprehensive profiling of Isoflavones, Phytosterols, Tocopherols, Minerals, Crude Protein, Lipid, and Sugar During Soybean (Glycine max) Germination. J Agric Food Chem 58:4970-6. DOI: 10.1021/jf100335j.

# **NOVEL CROPS REPORT**

Enhancing Profits and Sustainability in potato rotations using brown mustard (brassica juncea) for soil health and export grain production

### Principal Investigators: David Bell, Bell Crop Services (NB) & Steven Watts, Genesis Crop Services (PEI) / Dr. Aaron Mills AAFC Charlottetown PEI

Due to rapid expansion of wireworm as an economic pest in potatoes and the increase in the prevalence of potato early dying complex (EDC), growers have been increasingly interested in the use of biofumigant crops as part of the potato production system. Brown mustard (*Brassica juncea*) has seen the greatest increase in acres as growers attempt to capitalize on the potential bio fumigation properties of this plant. Although some studies have shown bio fumigation to be an effective alternative approach to pest control, brown mustard seed is expensive and the specific management of this crop as a biofumigant may be detrimental to soil structure. For example, when managed as a biofumigant, the use of secondary and tertiary tillage to incorporate brown mustard plants into the soil results in additional field passes and physical disturbance that would not have normally happened. The purpose of this project is to evaluate whether biofumigant effects of brown mustard can be realized if the plant is managed in ways other than through secondary tillage. This would include the use of flail mowing, or potentially harvesting the mustard seed as a cash crop.

Quantification of the level of reduction of wireworm damage in potato and overall return per acre following establishment of brown mustard (Brassica Juncea) for grain harvest versus plow-down in an Eastern Canadian potato rotation is the target of this work.

This work is being conducted both on private grower-cooperator sites as well as a site conducted by AAFC researchers at the Harrington Research Station (Dr. Mills).

Each season in Years 1-4 of the project, field sites are chosen for establishment of brown mustard management strips. All fields selected are in rotation with potatoes and follow a grain, oilseed, or forage crop for establishment with mustard (*B. Juncea*) and potatoes to follow. Three, approximately equal sized field-scale plots (strips) are established in each field to compare: 1) mustard allowed to mature for grain harvest, 2) mustard plowed down at peak flowering prior to full seed development, and 3) a check treatment of spring cereal. All plots and sections are accurately marked, and GPS mapped for accurate identification in the following year for surveying of potato quality.

This trial has been significantly challenged by the lack of adequate grower cooperators available to researchers. It has proved difficult, under the present economic and environmental conditions, to convince

producers to set aside land and time for research as the impacts of extreme weather wear away at their annual revenues and a global pandemic drive local demand but impede the ability to get workers to conduct day to day activities and supply the demand. In the first year, 2018, although some field sites were established, little was achieved in terms of results. In 2018, Season 1 mustard field sites were established at 3 locations in PEI and 1 location in New Brunswick. All fields selected were in rotation with potatoes and coming out of a grain, oilseed, or forage crop for establishment with mustard (*B. Juncea*) and potatoes to follow. No successful Season 1 field sites were established in PEI in 2019 due to lack of cooperating growers and grower mismanagement of plots; however, 2 sites were established in NB. All season 2(potato evaluations) were completed in 2019. 2020 saw a turnover in the management of these trials with the PEI grower sites being overseen by Steven Watts and his team at Genesis Crop Services. The NB sites were turned over to David Bell of Bell Crop Services in 2019 who continues to oversee and report on the trials.

#### **Results to Date:**

#### NB Site Report - Bell Crop Services

#### **Riverview Farms**

Mustard and control plots were established, mapped, and maintained in 2019. These crops included: corn, ryegrass, mustard chopped and plowed, and mustard harvested then plowed. Potatoes were planted in 2020 on the same sites and agronomic study completed. Project plan was for project collaborator, McCain Foods ltd. to harvest plots, complete yield, and qualitative data analysis.

Soil samples were taken on May 22 prior to planting potatoes. Samples were sent to PEI Potato Quality Institute lab and results shared on June 17. No significant difference was found among treatments for the verticillium ratings, Root Lesion Nematode (RLN) numbers were significantly less in the mustard plow down plots versus all other treatments, with rye control being next best control. Corn treatment showed largest RLN populations.

Research Site -Treatment	V. dahlia*	V. albo-atrum*	Root Lesion Nematode	
			(RLN)	
Riverview: mustard-harvest	2.5	0.75	19,301	
Riverview: mustard-plowed	2.5	0.5	5842	
Riverview: rye - control	2.5	0.75	8650	
Riverview: corn - control	2.6	0.87	22,259	

#### Table 1: Summary of soil test measurements for Riverview site spring 2020 NB ECODA 2020

\*average rating 1-3

Following potato crop establishment, fields were visited regularly during the growing season. Plots previously in corn rotation were observed to be the most healthy (full, green canopy) followed by ryegrass, then mustard harvested and then plowed, the least healthy-looking canopy was the mustard chopped mid-summer and plowed. Lack of rainfall caused all treatments to mature earlier than normal.

The grower cooperator harvested the trial field prior to the sampling of the 10-foot strips and therefore quality analysis was unable to be completed. Grower fortunately has access to yield data from the potato harvester

and total yields were able to be extrapolated for all treatments, with exception of the corn plot. Available yield data showed no significant difference in yield between treatments.

Research Site -Treatment	Yield (cwt/ac)
Riverview: mustard-harvest	239
Riverview: mustard-plowed	240
Riverview: rye - control	235

#### Brennan Farms

Mustard and control plots were established, mapped, and maintained in 2019. These crops included: barley under seeded to red clover, camelina, mustard chopped and plowed, mustard harvested and then plowed. Potatoes were planted in 2020 on the same sites and agronomic study completed. Project collaborator, McCain Foods ltd. harvested plots, completed yield and qualitative data analysis.

Soil samples were taken on May 22 prior to planting potatoes. Samples were sent to PEI Potato Quality Institute lab and results shared on June 17.

Research Site -Treatment	V. dahlia*	V. albo-atrum*	Root Lesion Nematode
			(RLN)
Brennan: mustard-harvest	2.3	1.2	6304
Brennan: mustard-plowed	2.25	2.0	4843
Brennan: barley - control	2.3	1.87	12,239
Brennan: camelina	2.1	0.8	10,354

#### **Table 3:** Summary of soil test measurements for Brennan site spring 2020 NB ECODA 2020

\*average rating 1-3

Although there was no significant difference in *V. dahlia* ratings among treatments, when it came to ratings for presence of *V. albo-atrum* the rating was significantly less, with 0 presence noted in 2 of the 4 plots sampled. Nematode numbers were significantly less in both mustard plots as compared to the barley control plot and camelina.

The Brennan field where the trial was located is usually a well-drained location and is considered dry field when moisture is abundant. This growing season developed into a very dry year and potato crops did not fare well. It was difficult to pick out the healthiest canopy, but barley plots did seem to be slightly greener than the mustard harvested and plowed as well as the mustard chopped mid-summer and plowed. The poorest stands of potatoes were those previously planted in camelina.

Sample potato 10 ft strips were harvested Sept 25 by McCain Foods field team. McCain team also completed quality analysis and grading of all potato samples, grading to fry standards.

 Table 2: Summary of potato yields for Riverview site 2020 NB ECODA 2020

Research Site -	Plants	Tubers	Total Yield	Market Weight	Value (\$/ac)
Treatment	(avg #)	(avg #)	(cwt/ac)	(cwt/ac)	
Brennan: mustard-	8	61	181.7	152	1,562
harvest					
Brennan: mustard-	7	70	176.3	131	1,329
plowed					
Brennan: barley -	7	60	176.8	152	1,618
control					
Brennan: camelina	6	47	114	86.5	874

The expected increase in yield from the reduction in verticillium and RLN did not happen in this field but excessively dry conditions this season could be a larger factor. Camelina plots showed significantly reduced yields and resulting market value than all other treatments regardless of environmental impact.

Year 1 mustard treatments alongside producer controls were established in 2020 at 3 new fields, which will be planted into potatoes in the spring of 2021. Plots were established at the following grower cooperator farms: Riverview Farms, Matt Brennan, and Vince Kilfoil. Riverview Farms and Brennan Farm both established the same crop rotations in 2020, just in different fields. Vince Kilfoil added a multi species plow down mixture as well as a plot of hemp for 4 different rotations.



Fig 1: Potato canopy of mustard plow down treatment NB ECODA 2020



Fig 2: Potato canopy of mustard-harvest treatment NB ECODA 2020

#### PEI Site Report – Genesis Crop Systems Inc

Two PEI commercial potato producers with a history of using brown mustard (BM) in their crop rotation programs agreed to participate in the project; Linden Lea (LL) Farms, Meadowbank and MWM Farms, Middleton (2 sites). Both farms produce commercial potatoes for the fresh and/or frozen processing industries and have past issues with wireworm (WW) damage in the potato crop.

The LL site featured a comparison of strips of BM planted for bio-fumigation (BMbio), BM planted for oilseed harvest (BMH) and field peas planted for grain harvest (P). The MWM sites both featured BM planted as above, plus a strip of barley planted for grain harvest (BH) and Sudan-Sorghum Hybrid (SSH) planted as a green manure plow down. Soil samples were collected in GPS referenced zones for each crop program at respective sites after crops were planted in early June to 8" depth using a Dutch Auger soil probe.

Soil samples were submitted to the PEI Analytical Laboratory for S3 soil analysis and to PEI Potato Quality Institute for quantification of Root Lesion Nematode (RLN) *Pratylenchus penetrans*, and *Verticillium dahliae* and *albo-atrum* (VW), two of the major causal agents associated with Potato Early Dying Complex (PED).

All fields were monitored regularly during the growing season. When BM reached a stage appropriate for biofumigation – early seed pod development (fig 1), a select strip was mowed with a flail mower and immediately incorporated and packed using the best available equipment from the respective farm. Figures 2 and 3 provide demonstrate this procedure.



Fig 1: Stage of BM growth just prior to bio-fumigation procedure – PEI ECODA 2020



Fig 2: Flail mowing BM PEI ECODA 2020



Fig 3: Incorporation and packing of freshly mowed BM PEI ECODA 2020

Bio-fumigated strips could lie fallow for a minimum of two weeks. Following this phase, a green manure crop was planted to provide soil protection for the remainder of the growing season (figure 4).



Fig 4: Reseeding BM bio-fumigation strip Aug 6 PEI ECODA 2020

Beyond this activity, sites received normal grower standard practice management and crops were harvested when mature.

#### RESULTS

Spring soil test information is provided below in table 1. Note that the soil samples VW data for the MWM 2 site were analyzed at the Agricultural Certification Services Lab in Fredericton, NB. This facility provides VW analysis reports in cells/gram of soil whereas the PQI reports using a scale of 0-4 where 0 = no VW presence; 4 = high DNA concentration of VW present.

There was some variation in values at each site. This is not necessarily unusual based on previous work examining in field variation in PEI potato fields.

			RLN	vw	vw
<u>Site</u>	<u>OM</u>	<u>pH</u>	per Kg dry Soil	<u>cells/g Soil</u>	Rating 1-3
MWM 1 Barley	3.3	6.1	0		2.5
MWM 1 BMbio	3.2	6.5	908		3
MWM 1 BMH	3.2	5.7	602		3
MWM 2 BM	2.6	6.5	593	4519	
MWM 2 BarH	2.5	6.6	1777	6850	
LL BMH	2.8	5.5	2399		3
LL BMbio	2.9	6.1	1197		2.5
LL Peas	3.1	5.2	9691		2.5

 Table 1: Summary of soil test measurements from MWM and LL sites spring 2020 PEI ECODA 2020

The 2020 growing season featured above average heat unit accumulation but unfortunately, significantly below average precipitation, especially in the BM site locations. Although there were no rain gauges at the sites, precipitation at the authors' location (Hampton - approximately midway between the sites) totaled 21mm, 36mm and 48mm for the months of June-August, respectively. This extended dry period not only resulted in low biomass production and reduced yields of harvested crops, but also the challenge of trying to execute an effective bio-fumigation program due to the difficulty in achieving good soil packing during the BM incorporation phase. It also provided challenging for good, reseeded crop germination and development following the fallow period – *fig 5*.

Most Island areas started receiving more "normal" rainfall in September – 79mm at Hampton, however, and resulted in reseeded crops filling in nicely for late fall ground cover – *fig* 6. Crop yields (table 2) were impacted by the dry conditions experienced throughout the growing season.



Fig 5: Dry soil conditions one week after reseeding PEI ECODA 2020



Fig 6: Reseeded oilseed radish/tillage radish LL site Oct 27 PEI ECODA 2020

<u>Site</u>	<u>Crop</u>	Yield lbs/acre
LL	Peas	2200
	BM	900
MWM 1	Barley	3520
	BM	770
MWM 2	Barley	3520
	BM	770

#### Table 2: Summary of harvested yields as reported by growers PEI ECODA 2020

#### AAFC DATA:

2020 was a challenge with this project. It was deemed a project of lower importance by executive in COVID assessment than other longer-term projects and it did not get the go-ahead until much later in the season. The lateness in planting resulted in reduced plant vigor which made the plants more susceptible to both biotic and abiotic stress. Limited access due to COVID-19 restrictions meant that the crops were not scouted as much as they should have been to deal with pests and disease in a timely fashion. As a result of this, flea beetles decimated an already stressed crop leaving little or no seed yield from most plots.

On the phytochemical analysis side of things, GSL analysis methodology has been refined and the team is currently working through the 2019 and 2020 samples. Samples were collected throughout the 2019 growing season, flash frozen in liquid nitrogen and stored at -80C. Plant tissues (above ground, below ground) and soil samples were processed separately, during plant phenological development and in response to agronomic treatments. 13 different analytical standards representing the most reported glucosinolates were sourced for quantitative analysis. Field samples were collected in 2020 for root and soils of experimental brown mustard plots, flash frozen in liquid nitrogen, and stored at -80C awaiting extraction and analysis. Upon return to the lab (Oct 2020), technical staff were assigned to optimize extraction parameters (dried vs. wet; sequential solvent extraction vs. monosolvent extraction; solvent composition). Highly sensitive MRM LC-MS methods were developed and optimized using commercial standards, and test samples were evaluated for glucosinolate diversity and levels.

#### 2021 Progress:

**PEI sites** – only 1 of the 3 season 1 (mustard + control) established sites in 2020 were planted into season 2 potatoes in 2021 due to farm strategy changes. To compensate for loss of site years, 5 season 1 sites were established in 2021 that will then be transitioned into season 2 potatoes in the final year of study (2022).

*NB sites* – All sites are continuing as planned. 3 new season 1 sites established for final rotation study.

**AAFC** - The unspent funds from 2020 were transferred to 2021 and 2022 growing seasons to accommodate the inclusion of a 2nd brown mustard variety (AAC Brown 18). This variety is growing in popularity in western Canada and regional production data are needed for Maritime Canada.

# Improving soil health and land-use efficiency through intercrops with pulses

#### Principal Investigator: Dr. Claude Caldwell, Dalhousie University

One of the challenges of growing some of these grain legumes, particularly dry peas, and beans, in our region is higher moisture levels and humidity resulting in increased incidence of disease and lodging. Lodging can cause significant quality and yield loss at harvest and increase the rate of disease. One way we can alleviate some of these issues is through intercropping with an upright cereal or oilseed crop. Intercropping is of increasing interest as it has the potential to reduce crop losses due to lodging and increase profitability on a per acre basis. Intercropping, especially with nitrogen fixing legume crops, has the potential to increase productivity of each individual crop through the optimization of nutrients, light and water as well as reduce incidences of pests and disease. While legumes are generally intercropped with cereals or grass species, brassica species respond more readily to nitrogen fertilizers, producing a greater impact when intercropped with a legume (Genarda et al. 2016). Previous studies intercropping legumes with brassicas, showed was substantial N transfer between legume and brassica, representing up to 30-50% of rhizodeposition (Cortes-Mora et al., 2010). This same study showed that the intercropped brassica and legume cultivars used different N sources, which enhanced the functional niche separation (Cortes-Mora et al., 2010). The ability for the two plants to maximize the available soil nutrition could in turn reduce the amount of fertility applications required, decreasing fertilizer costs to producers. Nitrogen is also important when considering the oil and protein of a crop, which are of utmost importance in the marketing of both the pulse as well as the brassica for high value markets such as food, feed, and fuel.

Mustard and camelina are two brassicas that are small emerging markets for our region. Currently most acres of mustard being grown in the Atlantic Provinces are being plowed under for use as green manure as a putative method of nematode and wireworm control. Mustard in the western region of Canada is a high value crop sold around the world for food ingredients, biopesticides, bioactive and industrial oil use. Camelina oil has several uses and benefits, including high value cosmetics (lotions, creams, soaps, haircare), replacement of marine(fish) oils with similar fatty acids, biofuel, nutraceutical, animal and aquaculture feed, and human consumption oil.

Land equivalent ratios (LER) values are the primary reference for the determination of the usefulness and return of an intercrop. An LER is calculated as the sum of the intercrop yield divided by the yield of the sole crop for each of the crops within the intercrop. A LER value of greater than one indicates that the intercrop is more productive than the sole crop. The projected outcome for this activity is a recommended pea intercrop that delivers a LER of greater than one, and therefore a higher return per acre versus a single crop, for producers in Eastern Canada for a single rotation year. This recommendation will also be based on high market potential and export value as well as improved soil health over the entire rotation of our agricultural lands.

The experimental trials are being led by Dr. Caldwell with multiple field sites in New Brunswick and Nova Scotia, chosen for variation in soil type and climate condition. Small plot experimentation is designed to evaluate the response of two brassica species camelina (C.sativa) and mustard (B.Juncea) intercropped with

peas. Control plots of each crop were established at recommended rates and seeded at different rates when intercropped. The plots were seeded by first drill seeding the peas and then followed by the brassica species seeded on top either drilled in rows between peas or via broadcast seeding. Intercrop treatments received nitrogen fertility at a rate of 60 kg N ha<sup>-1</sup>, while pea-only plots received 20 kg N ha<sup>-1</sup> and brassica plots received nitrogen at 100 kg N ha<sup>-1</sup>.



Figure 1: 2020 field site in Annapolis Valley, NS

#### Results to Date: Camelina: Pea Intercrop



Figure 2: Camelina yield response to intercrop treatment with peas



Figure 3: Pea yield response to intercrop treatment with camelina

Treatment	Camelina yield (kg/ha @ 8% moisture)	Pea yield (kg/ha @ 13% moisture)	LER
C 1.0 ; P 0.0	3314	0	1
C 0.0 ; P 1.0	0	3951	1
C 1.0 ; P 0.5	2518	1104	1.04
C 1.0 ; P 1.0	2115	2111	1.17
C 1.0 ; P 1.5	1777	2859	1.26
C 0.5 ; P 0.5	2348	1367	1.05
C 0.5 ; P 1.0	1881	1976	1.07
C 0.5 ; P 1.5	1440	2662	1.11
C 1.5 ; P 0.5	2957	846	1.11
C 1.5 ; P 1.0	2465	1605	1.15
C 1.5 ; P 1.5	1910	2075	1.1

Table	1:	I FR	Table	for	Camelina:	Pea	Intercron
Iavic	<b>-</b>		Iavie	101	Camenna.	гса	millercrop

The purpose of this trial was to find the optimal seeding ratio of camelina and peas to produce the highest LER for oil, protein, and per hectare profitability. Yield results show that the highest LER ratio was from 600 seeds/m<sup>2</sup> of camelina to 150 seeds/m<sup>2</sup> of peas, producing an LER of 1.26.

The intercropping of peas and camelina significantly affected yield and stand counts of both crops. In each case the recommended seeding rates of 600 and 100 seeds/m<sup>2</sup> for camelina and peas respectively had the highest mean yield but that was not statistically different from majority of the other 1.0 and 1.5 rate treatments. This agrees with the LER table, showing that there are several combinations of peas with camelina that can produce an LER of greater than 1.

In these trials, there was no significant effect of treatment on lodging. However, the two crops netted together with each other which tended to keep the intercrop standing.



Figure 4: Camelina-Pea Intercrop Plot

#### Mustard: Pea Intercrop

It is expected that intercropping of peas with mustard will result in the improvement of soil health because of the different soil nutrient requirement for each crop, different rooting pattern, which allow less competition and more soil profile break up, and brassica root exudate effects on soil pathogens.



Figure 5: Late June Mustard-Pea Intercrop Plot



**Figure 6:** Photo showing pea intercrop with mustard and sole pea growing. Mustard out competed peas during the season. During the growing season weeds were a major issue, hand weeding was done to mitigate the problem.



Figure 7: Pea Yield response to intercrop treatment with mustard 2020



Figure 8: Mustard Yield response to intercrop treatment with peas 2020

Mustard had a significant effect on the yield of peas, peas had no significant effect on the yield of mustard. The data shows that 50 mustard seeds/m<sup>2</sup> and 150 pea seeds/m<sup>2</sup> is the optimal Land equivalent ratio (LER) for yield.

Treatment	mustard yield (kg/ha @ 8% moisture)	Pea yield (kg/ha @ 13% moisture)	LER
m 1.0; P 0.0	1331	0	1
m 0.0; P 1.0	0	3191	1
m 1.0; P 0.5	1628	813	1.4
m 1.0; P 1.0	1265	1622	1.4
m 1.0; P 1.5	1243	1929	1.5
m 0.5; P 0.5	1434	1101	1.4
m 0.5; P 1.0	1099	1830	1.3
m 0.5; P 1.5	1307	2406	1.7
m 1.5; P 0.5	1526	527	1.2
m1.5; P 1.0	1287	1321	1.3
m1.5; P 1.5	1308	1419	1.4

#### Table 2: LER Table for Mustard: Pea Intercrop

Overall, the best intercrop ratio to obtain optimum total yield Land Equivalent Ratio (LER) for the camelina/pea intercrop was camelina 600 seeds/m2 and pea 150 seeds/m2 while the optimum yield LER for the mustard/pea was mustard 50 seeds/m2 and pea 150 seeds/m2. Work is continuing analysis for the oil, protein and per hectare profitability LER. Final calculations will be made using multiyear data following the 2021

harvest.

#### 2021 Progress:

2 sites established in Nova Scotia, one in Truro and one in Annapolis Valley region. Seeding challenges showed inconsistent emergence in some plots that will be considered in analysis. NB sites not established due to COVID challenges in Spring.

### Evaluation of diverse camelina germplasm to enhance profits and sustainability in Eastern Canadian rotations

#### Principal Investigator: Dr. Claude Caldwell, Dalhousie University

Canadian agronomic studies have proven that camelina can be successfully across Canada both East and West, with similar varieties performing well in both regions. Camelina will yield slightly higher in the West (1,500 - 2,000 kg/ha) but recent trials of some newer lines in Nova Scotia have yielded well over 1,000kg/ha. In drought conditions camelina will yield higher than canola and studies have shown that camelina has higher nitrogen use efficiency, especially under stress. Camelina oil has several potential uses and benefits, including high value cosmetics (lotions, creams, soaps, haircare), replacement of marine(fish) oils in aqua feed, biofuel, nutraceutical, animal feed, and human consumption oil. Camelina has most recently emerged in the agriculture biofumigant markets due to many varieties containing high glucosinolate levels contributing to control of soil born pests. Recent studies based out of Spain have shown effective control of several nematode species and are being applied in biofumigant mixes throughout the EU.

Research into maximizing returns and improving the quality of the camelina products through proper variety selection and diversification is important if we are going to innovate and develop the camelina industry in our region. Camelina certainly cannot replace our top performing cropping systems, but it certainly provides opportunities to maximize a return within our established rotations such as potatoes.

The primary objective of this research is to identify top performing camelina varieties for Eastern Canada for both soil enhancement as well as maximization of return in rotation.

#### **Results to Date:**

#### **Results/Observations:**

\*Means that do not share a letter are significantly different.

Variety	Mean	Fisher
CCE117	1603	А
CCE26	1413	А
CCE32	1008	В
CDI005	1004	В
CCE27	995	В

#### Table 1: Table of means for yield (kg/Ha @ 8% moisture)

#### **Table 2:** Mean table for lodging (Belgian lodging scale)

Variety	Mean	Fisher
CCE27	4.9	А
CDI005	4.7	А
CCE32	0.7	В
CCE117	0.5	В
CCE26	0.4	В

#### **Table 3:** Mean table showing plant height (cm)

Variety	Mean	Fisher
CCE117	85	А
CDI005	82	AB
CCE26	78	ВC
CCE27	78	ВC
CCE32	74	С

#### Table 4. Table of means and Fisher comparison for protein (%)

Treatment	Mean protein	Fisher
	(%)	Comparison *
3 – CCE 32	30.33	А
4 – CDI 005	29.95	A B
2 – CCE 27	29.79	A B C
1 – CCE 26	28.88	A B C
5 – CCE 117	28.58	С

**Table 5.** Table of means and Fisher comparison for oil (%) varietaldifference

Treatment	Mean oil (%)	Fisher
		Comparison *
4 – CDI 005	33.96	А
1 – CCE 26	33.15	A B
2 – CCE 27	33.12	A B
5 – CCE 117	32.33	ВC
3 – CCE 32	32.16	С

Overall, the growing season in 2020 was hotter and drier than 2019, in which there were no significant differences among the lines tested. In 2020, CCE 117 and CCE 26 demonstrated significantly higher yields than the other entries in the test, including the Canadian entry CDI 005. This may demonstrate their adaptation to drought and/or heat stress. Both lines demonstrated excellent straw strength compared to the Canadian check. CCE 117 had higher plant height, but this did not result in more lodging. Both lines seem well adapted to the Eastern Canadian growing conditions, especially as we experience warmer and drier conditions.

The results of the NIR analysis indicate that there are significant differences among the lines in terms of seed protein, seed oil content, and all the major fatty acids. While CCE 117 demonstrated high yield, it showed the lowest protein content and one of the lower overall oil contents. This can be the effect of dilution due to higher yield. CDI 005 demonstrated high protein and high oil content. In terms of fatty acid composition CCE 117 demonstrated high palmitic acid, oleic acid, and linoleic acid. Therefore, as expected, it had reduced amounts of linolenic acid and eicosadienioc acid. CDI 005 showed relatively high levels of oleic acid and linoleic acid.

#### 2021 Progress:

2 sites established in Nova Scotia, one in Truro and one in Annapolis Valley region. Good establishment and growth. Regular (and sometimes excessive) rainfall has meant the absence of any drought and some problem with weed control.

### **2021 COMMENCEMENT TRIALS**

Nutritional value and functionalities of non-genetically modified oilseeds for application in aquaculture feeds

Principal Investigator: CENTER FOR AQUACULTURE TECHNOLOGIES CANADA (CATC)

#### 2021 Progress:

Due to unforeseen challenges in the supply of the relevant seed for trialing the aquafeed study has been delayed to late 2021 for anticipated completion by the end of calendar 2022.